defined. In the Kyte and Doolittle scale[11], proline is considered a slightly hydrophilic amino acid, however the structural characteristic of the side chain of proline should impart a more hydrophobic character. This is reflected in the Akamatsu and Fujita scale[12], where the hydrophobic value is close to other hydrophobic amino acids, exactly between alanine and methionine.

Two different complementary peptides, reflective of these two possible hydropathic characteristics of proline, were synthesized. A slightly hydrophilic proline is best complemented by alanine, so the sequence ASA was chosen. A hydrophobic proline is best genetically complemented by arginine, and RTR was chosen. To increase the potential affinity for N-acetyl-PGP, complementary peptides were synthesized in multimeric forms, starting from a polylysine core, and spaced from the core with two glycines. The simple linear RTR and RTRGG sequences were also synthesized to verify the specificity of the RTR sequence in the multimeric peptides (Figure 1).

EXAMPLE 3

Peptide Synthesis and Isolation

Complementary peptides were synthesized using Solid Phase Peptide synthesis following Fmoc methodology on a 9050 Peptide synthesizer from Perseptive Biosystem. The linear peptides were synthesized using an Amide-polyethylene glycol graft polystyrene (PEG-PS) resin and *O*-pentafluorophenyl ester pre-activated amino acids. The branched peptides were synthesized starting from a Fmoc-Alanine-PEG-PS resin, with either one or two coupling cycles with Fmoc-K-Fmoc-OH activated with HATU/DIPEA. The following couplings were achieved using Fmoc-amino acids activated with HATU/DIPEA. The Fmoc deprotection reagent was 1% DBU, 1% Piperidine in dimethylformamide. The peptides were cleaved from the resins by adding 10 ml of trifluoroacetic acid (TFA)/phenol/thioanisol/H_2O/ethandithiol 93/2/2/2/1 and incubated at room temperature for 5 hours. The mixtures were filtered and the peptides precipitated in cold ethyl ether. The precipitates were collected and solubilized in H_2O for lyophilization.

All peptides were purified by reverse phase high performance liquid chromatography (RP-HPLC), using a Dynamax RP C18 (300x10mm i.d.), and equilibrated at 3 ml/min using a linear gradient from 5% CH_3CN to 60% CH_3CN in 0.1% TFA in 40 minutes. The fractions containing the peptide were acidified with 1 N HCl to help in the elimination of TFA, and lyophilized. Peptide identity was confirmed by time of flight matrix assisted laser desorption ionization mass spectroscopy. Purity was confirmed by analytical RP-HPLC.

For large-scale synthesis of N-acetyl-PGP, an alternative method was used to increase the yield of the product. In this method, the dipeptide *t*-Boc-PG was coupled to Pro-Merrifield resin using the dicyclohexylcarbodiimide/1-hydroxybenzotriazole procedure. After the removal of the N-terminal protection and acetylation using acetic anhydride, the peptide was cleaved from the resin using anhydrous hydrofluoric acid. The product was purified on a silica gel column using chloroform: methanol (90:10 v/v) as the eluent. Homogeneity was confirmed by RP-HPLC on a Vydac C18-analytical column equilibrated at a flow rate of 1.2 ml/min and eluted with a linear gradient from 0% to 30% acetonitrile in water